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TWO BACTERIAL PATHOGENS OF *HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE)

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Abstract.—Bacterial pathogens of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) were investigated. Two different pathogenic bacteria were isolated from unhealthy and dead larvae. They were identified as *Pantoea agglomerans* (Ewing and Fife) and *Alcaligenes piechaudii* Kiredjian et al. on the basis of fatty acid methyl ester (FAMEs) analysis and carbon utilization profiles by using Microbial Identification and Biolog Microplate Systems. Laboratory experiments carried out to determine insecticidal activities of these isolates showed that *Pantoea agglomerans* and *Alcaligenes piechaudii* have 95.0% and 98.75% mortality on third-instar larvae of *H. armigera,* respectively, after 14 days. This is the first study to demonstrate that *Pantoea agglomerans* and *Alcaligenes piechaudii* are pathogenic bacterial flora of *H. armigera.* These bacteria may have great potential for use in biological control of *H. armigera.*

Key Words: *Helicoverpa armigera,* bacterial pathogen, microbial control

The genus *Helicoverpa* consists of several pest species, and among them *Helcoverpa armigera* (Hübner) is one of the most economically important pests in Turkey. It is an especially notable pest of sorghum, maize, cotton, chickpeas, and tomatoes (Turkish Ministry of Agriculture 1995). Chemical substances utilized to control this pest have hazardous effects in the environment. As the use of chemical pesticides is a social issue, the objectives of nutrition, health, and environmental quality can be addressed more efficiently by the implementation of integrated pest management techniques (IPM) rather than through current crop protection practices (Norgard 1976).

Increasing problems with synthetic insecticides have spurred the search for alternative pest management strategies that would reduce reliance on synthetic insecticides. Biological control of plant pests is an alternative control method in lieu of chemical pesticides. Recently, several micro-organisms have been isolated and identified from insects as potential biological control agents (Weiser et al. 2002, Yaman 2003, Yaman and Radek 2003).

Studies on natural enemies of *H. armigera* have focused on pathogenic viruses (Tuan and Hou 1988, Teakle and Byrne 1989, Parnell et al. 1999, Narayanan 2002), *Bacillus thuringiensis* (Bt) (Navon et al. 1990), and microsporidia (Tsai et al. 2003). However, very little is known about other bacterial pathogens limiting its populations. We present results of a study on the isolation, identification, and insecticidal effect of two new pathogenic bacteria for *H. armigera* in Turkey.

Materials and Methods

Collection of *H. armigera* larvae.—During spring and summer 2001 and 2002, *H. armigera* larvae were collected from chickpea plants grown in the Agricultural Experiment Station in the vicinity of Erzurum, Turkey. The temperature range in the field was approximately 20–25°C. Dead and living larvae exhibiting characteristic disease symptoms (no feeding, slow moving, and color changes on the body) were selected and transported to the laboratory in sterile tubes within 1 h after collection. Dead larvae found in the field were put into sterile tubes to prevent possible contamination. All dead and unhealthy larvae collected were kept in a refrigerator for 2 h to be cooled then used for microbial isolation.

Isolation of bacterial strains and culture conditions.—After macroscopic examination, microscopic examination was done to be sure of bacterial infection. Dead larvae were surface sterilized in 70% alcohol (Poinar 1978). For this, a small vessel was filled with 10 ml of the disinfectant, the larva was put in it, and the vessel was corked and shaken for 2 minutes. The larva then was rinsed three times in sterile water in the same way in three clean vessels. After cutting the cuticle with sterile scissors, a drop of the fluid content was taken with an inoculating loop, diluted 100 times with sterile water, and spread on nutrient agar plates. Plates were incubated at 28°C to 37°C for 2–3 d. After the incubation period, plates were examined and bacterial colonies were selected (Lipa 1975, Thiery and Frachon 1997). Selected colonies were purified by subculture on plates. The two most prevalent colony types of bacteria were selected and purified on nutrient agar plate by subculturing. Bacterial strains were maintained for long-term storage in nutrient broth with 15% glycerol at −86°C for further tests.

Identification of bacterial strains.—All isolated bacterial strains were identified based on fatty acid profiles determined using the Microbial Identification System (Hewlett-Packard 6890A, Palo Alto, CA) with TSBA (Tripticose Say Broth Agar) database in the Sherlock Microbial Identification System software package (MIDI, Microbial ID, Inca, Newark, DE) and carbon substrate utilization fingerprints analyzed by the Biology GN and GP database with Microlog software in Biolog Microplate Systems (Biolog Inc., Hayward, CA). The isolates were stored at the Department of Plant Protection, Faculty of Agriculture, Ataturk University.

Bioassays of bacterial isolates.—Bioassays were performed on third-stage instars of 20 larvae of *H. armigera* for each bacterial isolate to determine their insecticidal activity. *Helicoverpa armigera* larvae damage chickpea by feeding on leaves and grains. Therefore, the larvae were fed with chickpea leaves and grains sprayed with the bacterial suspension (Dalmage 1981, McGuire et al. 1997). The bacterial isolates were grown in nutrient broth at 28°C overnight. The bacterial suspension for each pathogen was prepared in sterile water at the concentration of 10^8 CFU/ml and then immediately used for bioassay study. The control group was fed with chickpea leaves and grains sprayed with sterilized water. After 48 h, the larvae received fresh diet every 24 h. The larvae were tested for each bioassay during 14 d. The bacteria were readily re-isolated from dead larvae and identified as original isolates used for inoculation by MIS. All larvae tested were kept at 26°C and 60% RH on a 12:12 h photo regime. Observations were carried out daily and dead larvae were removed immediately. All bioassays were repeated 4 times on different days. Data were evaluated using Abbott's formula (Abbott 1925).

Data analysis.—Univariate using SPSS 11.0 software was used to determine if there was a statistically significant difference in insecticidal effect between the insecticidal activities and time. The results showed significant difference at $P < 0.05$ levels.

Table 1. Results of multiple comparison with mean and standard error of time. Values followed by different letters in the same column differ significantly at $P < 0.05$.

Time (Days)	N	*Pantoea agglomerans*		*Alcaligenes piechaudii*	
		Mean	SE	Mean	SE
5	4	36,25c**	4,732	43,75c**	5,543
10	4	77,50b**	6,614	83.75b**	3,145
14	4	95,00a**	2,041	98,75a**	1,250
Control	4	1,25d**	1,250	2.50d**	1,443

** $P < 0.05$.

Results and Discussion

Recent studies on natural enemies of *H. armigera* have been focused on pathogenic viruses (Tuan and Hou 1988, Teakle and Byrne 1989, Parnell et al. 1999, Narayanan 2002) and *Bacillus thuringiensis* Berliner and microsporidia (Tsai et al. 2003). In this study, we isolated two different nonspore-forming bacteria from living and dead larvae of *H. armigera.* Fatty acid analysis identified the bacterial pathogens as *Pantoea agglomerans* (Ewing and Fife) and *Alcaligenes piechaudii* Kiredjian et al. with similarity incidence of 53% and 89%, respectively. The identity of *Panoea agglomerans* at species level and *Alcaligenes piechaudii* at genus level was confirmed by Biolog GN Microplate with similarity incidence of 78% and 44%, respectively.

Different species of the genus *Alcaligenes* such as *A. recti, A. faecalis,* and *A. odorans* are found in insects (Lipa and Wiland 1972, Bucher 1981). Majumder et al. (1955) isolated *Bacillus thuringiensis* var. *thuringiensis* from *H. armigera.* Lipa and Wiland (1972) isolated four bacteria, *Aerobacter cloaceae* (Jordan), *Alcaligenes recti* (Packer and Vishniac), *Escherichia freundii* (Braak), and *Escherichia coli* (Migula) from *H. armigera,* but they did not test their pathogenicity on *H. armigera.* They gave an extensive list of bacteria and bacterial diseases recorded in Noctuidae. Our records are the first for *Pantoea agglomerans* and *Alcaligenes piechaudii* from Noctuidae. This study adds two pathogenic bacteria to the bacterial microflora of *H. armigera* and confirms that the bacterial microflora of *H. armigera* consists principally of nonspore-forming bacteria with one exception, *Bacillus thuringiensis* var. *thuringiensis.*

Results of the bioassays carried out for determining the insecticidal effect of the isolated bacteria showed that *Pantoea agglomerans* and *Alcaligenes piechaudii* have 95.00% and 98.75% mortality on the larvae of *H. armigera,* respectively, after 14 days (Table 1). Koch postulate studies showed that both of the bacterial strains caused similar disease symptoms on *H. armigera* larvae, which were lack of appetite, slowing and then failure in movement, and discoloration (blackish color) on the body. Lipa and Wiland (1972) tested pathogenicity of their bacteria isolated from *H. armigera* against third-instar larvae of *Agrotis c-nigrum* and *Agrotis segetum.* The highest pathogenic bacterium showed 67% and 92% mortality after two days for *Agrotis c-nigrum* and *Agrotis segetum,* respectively, and among them was *Alcaligenes recti* isolated from diseased larvae of *H. armigera* (Lipa and Wiland 1972). Our isolates showed high pathogenicity (95.00% and 98.75%) against *H. armigera* (Table 1). These are the first recorded non-spore-forming bacteria showing such high pathogenicity to *H. armigera.* We isolated them from the hemocoel of *H. armigera.* Potential pathogens multiply and cause death of insects after they penetrate the hemocoel in small numbers (Lipa 1975).

It is important that *Pantoea agglomerans* and *Alcaligenes piechaudii* have sufficient

insecticidal effects on the larvae of *H. armigera*, because biological control studies involving bacteria of *H. armigera* usually focus on *B. thuringiensis* (Navon et al. 1990). These bacteria can consequently be natural suppressors against *H. armigera* populations.

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